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A new model of carbon and phosphorus transfers in arbuscular mycorrhizas

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Summary

• Existing models of nutrient transfer in arbuscular mycorrhizal (AM) symbioses are inadequate as they do not explain the range of real responses seen experimentally. A computer simulation model was used to evaluate the novel hypotheses that mycorrhizal nutrient transfers were based solely on symbionts' internal needs, and that carbon and phosphorus transfers were quantitatively unlinked. To be plausible, simulated mycorrhizal plants would show a ±50% variation in weight vs non-mycorrhizal controls, with a normal response distribution (mimicking a real data set).
• One plant and one arbuscular mycorrhizal fungus (AMF) growing in a soil volume were simulated, using C, P and nitrogen nutrient cycling and stoichiometry. C- and P-exchange rates were independent and could be varied at will. The model was tested at realistic nutrient concentrations and a full range of nutrient exchange rates.
• The model showed –20% to +55% range in mycorrhizal plant weight distributed close to normal, suggesting that the hypotheses were plausible.
• The model suggests that theoretical assumptions about mycorrhizas should be reassessed. The model worked only because the symbionts possessed incomplete information on their partner and environmental conditions. Conventional cost–benefit models do not work under these circumstances, but both mutualistic and parasitic interactions were successfully simulated.

Key words: arbuscular mycorrhizas, ecological stoichiometry, modeling mycorrhizal interactions, plant–arbuscular mycorrhizal fungi (AMF) interactions.

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Introduction

The ubiquitous arbuscular mycorrhizas (AM) provide a number of services to the plants and arbuscular mycorrhizal fungi (AMF) that form them. However, most research has focused on the symbioses' role in nutrient exchange: the plant provides carbohydrates in return for soil nutrients, especially phosphorus (Smith & Read, 1997 and many others). The cellular, molecular and genetic processes involved in these nutrient transfers are still being elucidated (Harrison, 2005; Javot *et al.*, 2007), and there are substantial questions about the nutrient fluxes between the symbionts. While we do not yet have an experimental system in which these fluxes can be studied directly, we can model nutrient transfers based on information available from studies of organismal growth and nutrient uptake.

Although the model described here focuses on physiological interactions, it was stimulated by two independent research lines suggesting that the conventional view of nutrient fluxes – where nutrients are exchanged, and the costs and benefits of exchange are explored – might be inadequate. First, at high soil P levels, either in the soil or in the plant, some plants have been shown to exclude AMF from their roots (summarized by Smith & Read, 1997), presumably because the cost of obtaining P from the AMF outweighs the benefit of adding to the surplus P available, and the plant's resources are better spent obtaining limiting nutrients. Second, AMF can suppress plant growth (relative to nonmycorrhizal plants) (Johnson *et al.*, 1997 and

many others), and Klironomos (2003) demonstrated that such suppressive interactions occurred frequently among many combinations of plant and AMF species and ecotypes growing in monocultures at low but typical soil P concentrations. In this paper, the relative suppression of one symbiont's growth through the mycorrhizal interaction will be termed parasitism.

Together, these studies present a conundrum: if a plant can expel an AMF when it has sufficient P to forgo the carbon costs of the fungus, why does the plant not eliminate AMF under lower soil P conditions when the cost of the fungus outweighs its benefits to the plant? This does not seem to follow conventional cost–benefit logic, and no current model of nutrient fluxes explains it.

As an example, Fitter (2006) proposed a model for C and P fluxes to explain mutualistic nutrient exchanges wherein both symbionts benefit. His model is that P secreted by the AMF inside a root provokes a corresponding surge in growth and hexose secretion by the plant, which the fungus captures, and that, while AMF parasites exist, 'they would have to scavenge for sugars at the normal and typically low concentrations in the apoplast' (Fitter, 2006). This model may well be a good description of the process of nutrient exchange within a root when both P and hexose are available for exchange. However, it does not explain the conundrum described above. While this model can explain why AMF are excluded from roots at high soil P, it does not explain the prevalence of parasitism found by Klironomos (2003) and other studies. Clearly, another model is needed.

In creating a new model, one place to start is with the nature of P itself. It is weakly soluble, and is generally immobile and patchily distributed in soil (Brady & Weil, 2001). While both plants and AMF are able to obtain soil P in most soils, both organisms typically develop P depletion zones around roots or hyphae (Nye & Tinker, 1977; Clarkson, 1981). This contrasts with N and other soluble nutrients, which diffuse quickly (in some forms), and concentrations of which are therefore less patchy (Brady & Weil, 2001).

The immobility and patchiness of soil P has two implications for modeling. First, plants and AMF that need P cannot simply abide passively while P diffuses to them; rather, they have to grow actively through the soil to find P patches (through root extension), and take up P where they find it (through proliferation of fine roots). An organism that waits until P is found to expend C in acquiring it is doomed, because it will never grow beyond its initial P-depletion zone. Fitter's (2006) model misses this, because it focuses solely on the exchange of nutrients already obtained, not on the investment necessary to find and take up the nutrients. Under Fitter's model, a plant that is P-deficient, and that is not receiving P from an AMF, will expel that AMF, then presumably attempt to obtain P solely through root elongation out of the depletion zone. If this interpretation of Fitter's model is correct, it apparently runs counter to the basic observation that plants in low-P environments invest more rather than less heavily in AMF (Smith & Read, 1997).

The second implication of the nature of P is that plants and AMF have no way to assess the amount of P available in the soil beyond their depletion zones, in contrast to their ability to assess amounts of soil N. A plant in need of P cannot determine whether it would acquire more P through growing more roots or through sending more C to a mycorrhiza.

To accommodate the immobility and patchiness of P, a new conceptual model was created. It starts with the assumption that a plant with growth limited by P has to invest to acquire more P. That investment can be in biomass allocated to root growth, or carbohydrates (simplified here to carbon) given to an AMF. Because the availability of P is unknown, the plant cannot make a cost–benefit calculation to determine which investment will have a better return, so it may invest in both to 'hedge its bets'. The AMF similarly needs to invest in hyphal growth to acquire soil P. However, it also needs to acquire C through the mycorrhizal interaction, as it has a negligible ability to acquire C from the soil. Fortunately, the plant will provide C so long as it needs P, so the fungus can be supported in acquiring new P sources and passing some to the plant. Similarly, the P sent by the AMF to its photobiont is also an investment. An AMF cannot assess the photosynthetic ability of its photobiont, as it can only monitor the amount of C available to it at the mycorrhizal interface, rather than the light environment of the plant. Thus the AMF cannot make a cost–benefit calculation of the optimal amount of P to exchange in return for C.

The term 'investment strategy' is assumed to be the result of gene action, not conscious 'choice' on the part of the plant or AMF. However, it is a useful idea. In this model, C and P fluxes of these two nutrients are decoupled, rather than being instantaneously exchanged. The organisms are sending surplus nutrients (plant C, AMF P) to their partner at some rate based on internal need and, if the relationship is mutualistic, each partner receives sufficient amounts to live and reproduce.

The model put forward here solves the conundrum. A plant that has sufficient P will not invest any C in the AMF, resulting in diminishment or loss of mycorrhizas. However, under P-limiting conditions the plant will invest in mycorrhizas and/or more roots, simply because it needs to acquire more P. AMF parasitism of plants in glasshouse pot conditions should be common because, with a limited amount of P available in the system, the amount of P residing in AMF biomass is unavailable to the plant, resulting in a smaller plant. In the wild, either a plant or AMF can be parasitized, but this can be seen as a failed investment. The C sent to a mycorrhiza simply did not result in limiting P being supplied back.

This model seems plausible, but it needs to be tested. As there is no experimental system within which to monitor C and P fluxes across a mycorrhizal interface over time and under varying soil conditions, we constructed a physiological model of one plant and one AMF in a defined volume of soil,

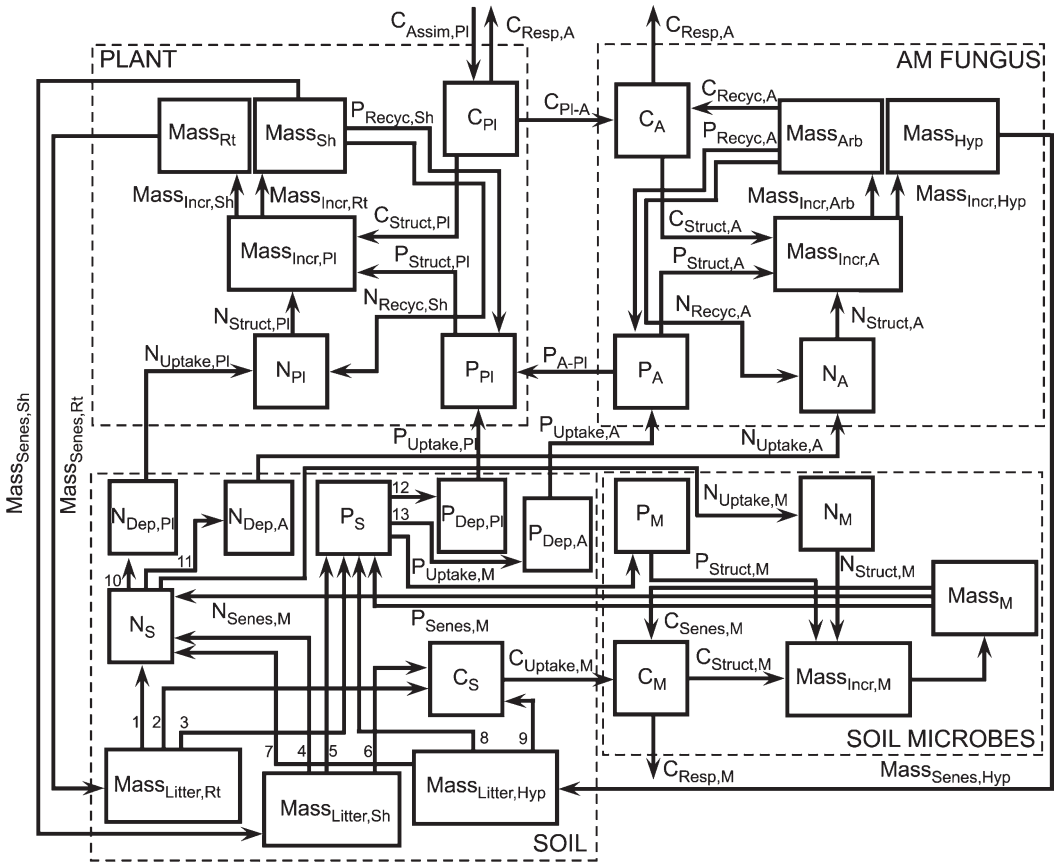

Fig. 1 Model schematic. Arrows show flows (movement or transformation of nutrients); boxes show pools. Labels are explained under Description. 1, $N_{Min,Rt}$; 2, $C_{Min,Rt}$; 3, $P_{Min,Rt}$; 4, $N_{Min,Sh}$; 5, $P_{Min,Sh}$; 6, $C_{Min,Sh}$; 7, $N_{Min,Hyp}$; 8, $P_{Min,Hyp}$; 9, $C_{Min,Hyp}$; 10, $N_{Inflow,Pl}$; 11, $N_{Inflow,A}$; 12, $P_{Inflow,Pl}$; 13, $P_{Inflow,A}$.

matching the common experimental setup. C and P fluxes were independent and partially controlled by initial settings, and the organism's decisions about nutrient and biomass allocations were based solely on internal needs, not cost–benefit equations. The output from this model was tested against the range of plant responses to AMF seen by Klironomos (2003) under similar experimental conditions. As Klironomos's data set involved many plant and AMF species, this test also explored how much of the variation seen could be explained by variations in nutrient exchange. A poor match would suggest that the physiological or morphological diversity of the organisms studied by Klironomos explained his results, whereas a good match would suggest that the mechanisms of nutrient transfer played a major role in determining the results.

Description

This model simulates a common experimental setup: one plant and one AMF growing in a fixed volume of soil. The plant and fungus are modeled in similar ways. The simulation uses only three nutrients: C, N and P (Fig. 1). Where the plant (subscript Pl) takes up C through its shoot (Sh) and N and P through its roots (Rt), the fungus takes in C through its 'arbuscules' (Arb, technically, the intraradical hyphae), and N and P through its 'hyphae' (Hyp, the extraradical hyphae). Nutrients are taken up by the organisms from the soil and incorporated into biomass, which in turn is lost by senescence back into litter. The litter is broken back into its component nutrients by soil microbes, which also take up nutrients, grow and senesce. The plant transfers C to the AMF, while the AMF transfers P to the plant; both compete for soil N, and compete with soil microbes for soil N and P. In the model, N and P cycles are closed (total recycling; Chapin, 1991; Lambers *et al.*, 1998), while the C cycle is open, with C being assimilated by the plant and respired by plant, AMF and soil microbes. Nitrogen is not exchanged between the partners, but it serves two useful purposes: it is important in decomposition, because the role of C : N in litter decomposition is well known compared with C : P, and because biomass incorporates N, it serves as a useful mathematical check throughout the model (for instance, ensuring that total biomass does exceed the amount possible based on system N).

In this model, variables typically have the form '$A_{B,C}$' (Fig. 1). 'A' is typically, although not exclusively, a nutrient (C,

N, P or Mass for biomass). Subscript 'B' describes the flow or pool, such as 'S' for soil, 'uptake', 'assim' for assimilation, 'senes', for senescence, etc. Finally, subscript 'C' denotes the organism, part or pool, such as 'Pl' for Plant, 'A' for AMF, or 'M' for soil microbes. Thus $N_{Uptake,Pl}$ is the N taken up ('Uptake') by the plant ('Pl').

The two key features in this model involve the nutrient exchange between plant and AMF. First, C and P exchange ($C_{Pl\text{-}A}$ and $P_{A\text{-}Pl}$) are not coupled. The rates of P and C transfer are not linked, but rather are governed by the surface area of the arbuscules, the need for P (plant) or C (AMF) in the donor, and an exchange-efficiency term. The second feature is the C- and P-exchange efficiency terms (C_{ExEff} and P_{ExEff}), which are unitless coefficients that can be set anywhere from 0.0 to 1.0. The C_{ExEff} and P_{ExEff} terms make a number of states possible. At a (C_{ExEff}, P_{ExEff}) of 1, 1, nutrients are flowing at their maximum rate, while at (0, 0) no nutrients are flowing. These are analogous to mycorrhizal and nonmycorrhizal states in experiments. Furthermore, at (1, 0), C is flowing to the fungus, but no P is flowing back, simulating the fungus acting as a parasite. However, the reverse (0, 1) should be the same as (0, 0). As the fungus obtains its entire C from the plant, it should fail to grow under these conditions, resulting in a nonmycorrhizal system. Note that other states, such as (0.5, 0.5), are also possible.

The key features were deduced from two results of Klironomos (2003): first, in a system where many plant species were grown with a single AMF species, the resulting final biomass curve (relativized to the biomass of nonmycorrhizal species) was normal with a mean of zero; and second, in an experiment where different ecotypes of plants and AMF were cross-matched in a fully replicated block experiment. In this case there was no pattern to the results. One plant species might grow relatively larger with one AMF ecotype, but it would show a growth reduction with a different ecotype of the same AMF species, and the results could be reversed for another plant species or ecotype (Klironomos, 2003).

Both results favor decoupling C and P transfers. The fact that 50% of the plants were smaller than their nonmycorrhizal conspecifics suggests that C and P were not being exchanged in any constant C : P ratio. If there was a constant exchange ratio, it would be difficult to find mycorrhizal plants that were smaller than their nonmycorrhizal conspecifics, which result from no nutrient exchange at all. Similarly, the lack of pattern in the ecotype-based responses, and the frequent parasitic responses seen there, cannot be explained by direct nutrient exchange at a constant ratio.

Both results, particularly those from the ecotype experiment, also favor the C- and P-exchange efficiency terms. The range of results in the first experiment, coupled with the lack of pattern in the second, suggest that there was a wide range of C- and P-transfer rates. Lacking more definite information, continuously variable exchange coefficients are the simplest way to model this complexity.

One question is how the key features relate to current findings in mycorrhizal genetics. The normal curve results suggest that many genes could be at work, as single gene controls would be more likely to produce a large plant/small plant bimodal response. There is evidence for multiple P transporters in plants that are uniquely involved in the AM symbiosis (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Versaw *et al.*, 2002; Maeda *et al.*, 2005, 2006; Nagy *et al.*, 2005, 2006; Javot *et al.*, 2007), so it is plausible to assume that many genes are responsible. However, all these papers focus on the effects of having functional vs nonfunctional copies of single genes, and such single-gene responses are definitely not underlying Klironomos's (2003) results. For example, if one ecotype had a defective gene, all mycorrhizas formed with that ecotype would effectively be nonmycorrhizal. This was not seen. The important point is that both key features were deduced from Klironomos (2003), and it will be fascinating to see whether this model is supported when researchers determine the biology of nutrient exchange across the mycorrhizal interface.

As with any simulation, this model embodies a number of assumptions. The three most important are nutrient stoichiometry, nutrient cycling and goal-seeking optimality behavior by the plant and AMF. Even more fundamentally, the model is built on weights and volumes, rather than moles and concentrations. While some authors suggest that concentrations are proper, especially when using stoichiometry (Sterner & Elser, 2002), in this model, weights were simpler. Indeed, volumes were only needed for calculating nutrient uptake from soil.

Nutrient stoichiometry is the assumption that plant and AMF biomass contain a relatively constant ratio of nutrients. In model terms, biomass has a constant C : N : P. This is supported by data for both plants and fungi (Sterner & Elser, 2002), with the major caveat that both are capable of luxury consumption. Basically, luxury consumption is the uptake of nutrients beyond the immediate needs for forming new biomass, and in plants (and probably in fungi) surplus nutrients appear to be stored mostly in vacuoles (Leigh & Wyn-Jones, 1985; Sterner & Elser, 2002). In the model, nutrients are handled in a straightforward way. All nutrients are divided into two pools, 'nonstructural' and 'biomass.' Nutrients are assimilated into the nonstructural pool, and biomass is formed by taking nutrients out of the nonstructural pool in defined C : N : P ratios. When biomass is lost, it decomposes into its original nutrients, based again on the underlying C : N : P ratios. Finally, the maximum amount of the nonstructural pool is assumed to equal to the biomass pool. For example, if plant biomass contained 10 g N, then the maximum nonstructural N would be 10 g.

Nutrient cycling is the second assumption of the model. Available data suggest that 80% of soil nutrients taken up by plants are recycled within the local ecosystem from the senesced tissues of that or other organisms (Chapin, 1991;

Lambers *et al.*, 1998). Arguably, 100% recycling is closer to 80% than is 0%, so in this model, N and P are closed-cycled, whereas C is open-cycled. While total recycling is not strictly realistic, it avoids the bigger problem of nutrient gains and losses from the system unduly influencing the results. More importantly, closed cycles for N and P meant that the amounts of these nutrients in the model stayed constant over time. This characteristic proved vital for error checking during the model's development.

The third and final assumption is that the organisms seek the goal of maximizing growth through allocating biomass to acquire the nutrients limiting growth at any particular time. For example, if the plant is limited by N, it would allocate more to root growth, whereas if it was limited by C, it would allocate more to shoot growth, and P limitation would provoke increased C allocation to mycorrhizas and roots. Mechanistically, the limiting nutrients were determined by comparing the nutrients in the nonstructural pools to organismal C : N : P biomass requirements. This part of the model was inspired by Thornley's work (especially Thornley, 1995, 1997).

Biomass and volumetric controls

Plant and AMF Plant and AMF biomass are modeled in similar ways. Plant biomass ($Mass_{Pl}$) is subdivided into shoot and root biomass ($Mass_{Sh}$ and $Mass_{Rt}$), and AMF biomass ($Mass_A$)is composed of arbuscular ($Mass_{Arb}$) and extraradical hyphae ($Mass_{Hyp}$) (Table 1). In both organisms, the amount of biomass added ($Mass_{Incr,Pl}$, $Mass_{Incr,A}$) is the minimum of a relative growth rate (RGR_A, RGR_P) and minimum growth rates ($G_{Min,Pl}$, $G_{Min,A}$) based on limiting nutrients and stoichiometric ratios (Table 1). The plant's biomass : C : N : P ratio is 1 : 3 : 10 : 100, whereas the AMF ratio is 1 : 2 : 10 : 100 (Verhoeven *et al.*, 1996; Sterner & Elser, 2002). G_{Min} is constrained by the minimum concentration of C, N or P, based on biomass stoichiometry. Maximum potential growth rate ($G_{Max,Pl}$, $G_{Max,A}$) is used to determine what nutrient is most limiting, in order to allocate future growth, as explained below (Table 1). $Mass_{Incr,A}$ is more complex than $Mass_{Incr,Pl}$, because root mass ($Mass_R$) sets an upper limit for arbuscule mass ($Mass_{Arb}$), here arbitrarily modeled as 20% of $Mass_R$. Without the logistic term in $Mass_{Incr,A}$, arbuscular biomass could exceed the biomass of the root in which it is growing.

Biomass increase depletes nonstructural pools of C, N, and P. Only the limiting nutrient is depleted at any step, so persistent nonstructural nutrient pools can occur for nonlimiting nutrients. When any nonstructural pool reaches an upper limit (see below), uptake of that nutrient stops.

Once biomass has increased, the increase is immediately and entirely parceled into mass increase in shoot and root ($Mass_{Incr,Sh}$ and $Mass_{Incr,Rt}$), based on stoichiometric growth ratios (Table 1). Root mass allocation ($Mass_{Incr,Rt}$) is a somewhat complex equation. Where N_{Pl} or P_{Pl} constrains growth, especially where both are low, the second term in $Mass_{Incr,Rt}$ will be close to 1, and most of the mass increase will be allocated to the root. Where C is limiting, the second term of $Mass_{Incr,Rt}$ will be closer to zero, and most of the biomass will be allocated to the shoot, because shoot mass allocation ($Mass_{Incr,Sh}$) is merely the difference between total mass increase ($Mass_{Incr,P}$) and root mass increase. The equations for hyphal mass increase ($Mass_{Incr,Hyp}$) and arbuscular mass ($Mass_{Incr,Arb}$) increase are equivalent (Table 1).

Roots and hyphae are modeled as cylinders of fixed radius and variable length, which are used to calculate surface areas (SA_{Rt}, SA_{Hyp}; Table 1). All these equations ignore the complex geometry of real roots, hyphae and arbuscules, along with the related questions of how these structures grow, branch and increase in radius. Experiments in modeling these structures more realistically demonstrated that more realistic modeling of root and AMF geometry also required more realistic models of soil heterogeneity and nutrient patchiness in order to achieve believable results. That experience suggested that such complex models should be reserved for experimental systems in which the benefits of complexity justify the costs of obtaining the data to support it. In this proof-of-concept model, such complexity was counterproductive.

All plant and fungal organs have a lifespan, after which they senesce and are lost. For shoots and arbuscules, N and P are recycled back into the nonstructural pool, either partially or wholly. There is no evidence for nutrient recycling in roots or extraradical hyphae, so all nutrients are lost to the soil when these organs senesce (Lambers *et al.*, 1998). In the current model, senescence is simulated with a delay function. The mass increase to an organ on a day is lost through senescence ($Mass_{Senes,Sh}$, $Mass_{Senes,Rt}$, $Mass_{Senes,Arb}$, $Mass_{Senes,Hyp}$) at a set period later (Table 1). While this is an imperfect solution, it allows organ lifespans to be varied at will. In this model, shoot and root senescence were set at 180 d, arbuscular and hyphal senescence at 30 d.

Biomass from shoots, roots and hyphae senesces into litter ($Mass_{Litter,Sh}$, $Mass_{Litter,Rt}$, $Mass_{Litter,Hyp}$) (Table 1). Senesced arbuscule biomass is recycled into nonstructural nutrients in the fungus, as the arbuscules are inside the roots. Litter is mineralized ($Mass_{Min}$) at a constant rate (DepolyRate) as a function of microbial biomass ($Mass_M$) (Table 1). The C, N and P are released to soil pools (C_S, N_S, P_S) as a function of mass stoichiometry. For example, C input to soil from shoot litter is 1/3 of $Mass_{Senes,Sh}$. This is detailed below.

Soil microbial biomass Soil microbial biomass ($Mass_M$; Table 1) has a C : N : P ratio of 120 : 10 : 1. Biomass increase ($Mass_{Incr,M}$) is based on a C : N ratio of 12 : 1 (Brady & Weil, 2001; Table 1). The persistence of $Mass_M$ depends entirely on microbial C (C_M). So long as C comes in, the microbes grow. However, when C is inadequate to maintain microbial respiration ($C_{Resp,M}$), the microbial pool cannibalizes itself, losing biomass and respiring the C made available from this

Table 1 Plant, arbuscular mycorrhizal fungal (AMF) biomass and volumetric equations, variables, and constants

Equation	Units
$CNRatio_M = (C_M + (12Mass_M))/(N_M + Mass_M)$	
$Density_{Arb} = 0.2$	g cm^{-3}
$Density_{Hyp} = 0.2$	g cm^{-3}
$Density_{Rt} = 0.2$	g cm^{-3}
$DepolyRate = 0.8$	g g^{-1}
$G_{Max,A} = (2C_A,100N_A,1000P_A)$	g g^{-1}
$G_{Max,Pl} = \max(3C_{Pl},100N_{Pl},1000P_{Pl})$	g g^{-1}
$G_{Min,A} = \min(2C_A,100N_A,1000P_A)$	g g^{-1}
$Length_{Arb} = (Mass_{Arb}/Density_{Arb})/\pi Radius^2_{Arb}$	cm
$Length_{Hyp} = (Mass_{Hyp}/Density_{Hyp})/\pi Radius^2_{Hyp}$	cm
$G_{Min,Pl} = \min(3C_{Pl},100N_{Pl},1000P_{Pl})$	g g^{-1}
$Mass_A = Mass_{Arb} + Mass_{Hyp}$ (initially 0.002)	g
$Mass_{Arb}(t) = Mass_{Arb}(t - dt) + (Mass_{Incr,Arb} - Mass_{Senes,Arb})dt$ (initially 0.001)	g
$Mass_{Hyp}(t) = Mass_{Hyp}(t - dt) + (Mass_{Incr,Hyp} - Mass_{Senes,Hyp})dt$ (initially 0.001)	g
$Mass_{Incr,A}(t) = Mass_{Incr,A}(t - dt) + (Mass_{Incr,A} - Mass_{Incr,Arb} - Mass_{Incr,Hyp})dt$	g
$Mass_{Incr,Arb} = Mass_{Incr,A} - Mass_{Incr,Hyp}$	g d^{-1}
$Mass_{Incr,Hyp} = Mass_{Incr,A}((100N_A + 1000P_A)/(2CA + 100N_A + 1000P_A))$	g d^{-1}
$Mass_{Incr,M} = \min((C_M - C_{Resp,M}),12N_M,120P_M)$	g d^{-1}
$Mass_{Incr,Rt} = Mass_{Incr,Pl}(1 - (100N_{Pl} + 1000P_{Pl})/(3C_{Pl} + 100N_{Pl} + 1000P_{Pl}))$	g d^{-1}
$Mass_{Incr,Sh} = Mass_{Incr,Pl} - Mass_{Incr,Rt}$	g d^{-1}
$Mass_{Litter,Hyp}(t) = Mass_{Litter,Hyp}(t - dt) + (Mass_{Senes,Hyp} - Mass_{Min,Hyp})dt$ (initially 0)	g
$Mass_{Litter,Rt}(t) = Mass_{Litter,Rt}(t - dt) + (Mass_{Senes,Rt} - Mass_{Min,Rt})dt$ (initially 0)	g
$Mass_{Litter,Sh}(t) = Mass_{Litter,Sh}(t - dt) + (Mass_{Senes,Sh} - Mass_{Min,Sh})dt$ (initially 0)	g
$Mass_M(t) = Mass_M(t - dt) + (Mass_{Incr,M} - Mass_{Senes,M})dt$ (initially 0.012)	g
$Mass_{Min,Hyp} = Mass_{Lit,Hyp}Mass_M DepolyRate$	g d^{-1}
$Mass_{Min,Rt} = Mass_{Lit,Rt}Mass_M DepolyRate$	g d^{-1}
$Mass_{Min,Sh} = Mass_{Lit,Sh}Mass_M DepolyRate$	g d^{-1}
$Mass_{Pl} = Mass_{Rt} + Mass_{Sh}$ (initially 0.2)	g
$Mass_{Rt}(t) = Mass_{Rt}(t - dt) + (Mass_{Incr,Rt} - Mass_{Senes,Rt})dt$ (initially 0.1)	g
$Mass_{Senes,Arb}(t) = 0.75Mass_{Incr,Arb}(t - 30) + \max(0,(Mass_{Arb} - 0.2Mass_{Rt}))$ (initially 0)	g
$Mass_{Senes,Hyp}(t) = 0.75Mass_{Incr,Hyp}(t - 30)$ (initially 0)	g
$Mass_{Senes,Rt}(t) = Mass_{Incr,Rt}(t - 180)$ (initially 0)	g
$Mass_{Senes,Sh}(t) = Mass_{Incr,Sh}(t - 180)$ (initially 0)	g
$Mass_{Senes,M} = Max((0.5Mass_M - 0.0012 - C_{Resp,M}),0)$	g
$Mass_{Sh}(t) = Mass_{Sh}(t - dt) + (Mass_{Incr,Sh} - Mass_{Senes,Sh})dt$ (initially 0.1)	g
$RGR_A = 0.1$	g g^{-1}
$RGR_{Pl} = 0.1$	g g^{-1}
$SA_{Arb} = 2Mass_{Arb}/(Density_{Arb}Radius_{Arb})$	cm^{-2}
$SA_{Hyp} = 2Mass_{Hyp}/(Density_{Hyp}Radius_{Hyp})$	cm^{-2}
$SA_{Rt} = 2Mass_{Rt}/(Density_{Rt}Radius_{Rt})$	cm^{-2}
$V_{DepN,A} = \min(\pi Length_{Hyp}((1.3 + Radius_{Hyp})^2 - Radius^2_{Hyp}),V_s)$	cm^3
$V_{DepN,Pl} = \min(\pi Length_{Rt}((1.3 + Radius_{Rt})^2 - Radius^2_{Rt}),V_s)$	cm^3
$V_{DepP,A} = \min(\pi Length_{Hyp}[(0.0018 + Radius_{Hyp})^2 - Radius^2_{Hyp}],V_s)$	cm^3
$V_{DepP,Pl} = \min(\pi Length_{Rt}[(0.0018 + Radius_{Rt})^2 - Radius^2_{Rt}],V_s)$	cm^3
$V_S = 10\,000$	cm^3

loss. N and P lost in this way return to the soil pool (N_S and P_S), where they may be taken up by either the microbes or the plant or fungus.

Soil volume and nutrient concentrations While nutrient cycling is done almost entirely on a mass basis, nutrient-uptake equations are based on nutrient concentrations in the soil. Here the soil volume (Vol_S) is divided into five overlapping compartments: the predefined soil volume (10 000 cm^3), and nutrient depletion zones for N and P ($Vol_{Dep,N,Pl}$, $Vol_{Dep,P,Pl}$, $Vol_{Dep,N,A}$, $Vol_{Dep,P,A}$) for plant roots and fungal hyphae (Table 1). Each of the four depletion zones

Table 2 Plant, arbuscular mycorrhizal fungal (AMF), and microbial carbon equations, variables, and constants

Equation	Units
$C_A(t) = C_A(t - dt) + (C_{Pl\text{-}A} + C_{Recyc,A} - C_{Resp,A} - C_{Struct,A})dt$ (initially $0.1Mass_A$)	g
$C_{Assim,Pl} = NAR\ SLA(LMR\ Mass_{Sh})^{0.75}(1 - 3C_{Pl})/Mass_{Pl}$	g d^{-1}
C_{ExEff} varies from 0 to 1	unitless coefficient
$C_M(t) = C_M(t - dt) + (C_{Uptake,M} + C_{Senes,M} - C_{Resp,M} - C_{Struct,M})dt$ (initially 0.0012)	g
$C_{Min,Hyp} = Mass_{Min,Hyp}/2$	g d^{-1}
$C_{Min,Rt} = Mass_{Min,Rt}/3$	g d^{-1}
$C_{Min,Sh} = Mass_{Min,Sh}/3$	g d^{-1}
$C_P(t) = C_{pl}(t - dt) + (C_{Assim,Pl} - C_{Resp,Pl} - C_{Pl\text{-}A} - C_{Struct,Pl})dt$ (initially $0.1Mass_{Pl}$)	g
$C_{Pl\text{-}A} = 0.000018SA_{Arb}C_{ExEff}((G_{Max,Pl} - 1000P_{Pl})/(G_{Max,Pl} - G_{Min,Pl}))(1 - (2C_A/Mass_A))$	g d^{-1}
$C_{Recyc,Arb} = Mass_{Senes,Arb}/2$	g d^{-1}
$C_{Resp,A} = 0.014Mass_A + (C_A - 0.2Mass_A)$	g d^{-1}
$C_{Resp,M} = 0.2Mass_M$	g d^{-1}
$C_{Resp,Pl} = 0.014Mass_{Pl}$	g d^{-1}
$C_S(t) = C_S(t - dt) + (C_{Min,Sh} + C_{Min,Rt} + C_{Min,Hyp} - C_{Updake,M})dt$ (initially 1)	g
$C_{Senes,M} = Mass_{Senes,M}$	g d^{-1}
$C_{Struct,A} = 0.5min(RGR_A, Mass_A, G_{Min,A})((1 - Mass_{Arb})/(0.2Mass_{Rt}))$	g d^{-1}
$C_{Struct,M} = Mass_{Incr,M}$	g d^{-1}
$C_{Struct,Pl} = 1/3min(RGR_{Pl}, Mass_{Pl}, G_{Min,Pl})$	g d^{-1}
$C_{Uptake,M} = 1.2Mass_M$	g d^{-1}
LMR = 0.49	g g^{-1}
NAR = 9.0	g m^{-2} d^{-1}
$SA_{Arb} = 2Mass_{Arb}/(Density_{Arb}Radius_{Arb})$	cm^{-2}
SLA = 0.0407	m^2 g^{-1}

is a cylindrical torus: the inner edge is the surface of the root or hypha, the radius is defined as the distance that an atom of N or P can travel in one day (1.3 and 0.0018 cm, respectively), and the length of each torus is the length of the root or hypha. The depletion zone equations specify the amount of nutrients available to the organisms each day, and limit the maximum depletion zone to the entire soil volume (Vol_S) (Table 1).

Carbon

Plant carbon The initial nonstructural C pool for the plant is $C_{Pl} = 0.1\ Mass_P$. The plant takes in C through photosynthesis ($C_{Assim,Pl}$), using net assimilation rate (NAR), specific leaf area (SLA), leaf mass ratio (LMR, ratio of leaf to shoot), $Mass_{Sh}$ and $Mass_P$ (Table 2). The assimilation equation is based on a standard plant growth equation (Lambers *et al.*, 1996), modified by a 0.75 scaling exponent (following Brown *et al.*, 2004) that scales photosynthesis to shoot mass without explicitly modeling leaf and stem growth and architecture. The final logistic term is based on plant mass stoichiometry, and its function is to shut off assimilation if the plant contains a C surplus.

Assimilated C (C_{Pl}) is allocated, in order, to respiration ($C_{Resp,Pl}$), exchange to AMF ($C_{Pl\text{-}A}$), and finally to biomass ($C_{Struct,Pl}$) (Table 2). The C transferred as $C_{Pl\text{-}A}$ is a function of arbuscule surface area (SA_{Arb}), C-exchange efficiency C_{ExEff}, and the plant growth rates $G_{Max,Pl}$ and $G_{Min,Pl}$. Some C ($C_{Struct,Pl}$) is incorporated into biomass based on minimum, maximum and relative growth rates, and any C not used remains in the nonstructural pool (C_P) (Table 2).

AMF carbon The initial nonstructural C pool for the fungus is $C_A = 0.1\ Mass_A$, as with the plant (Table 2). The fungus obtains all of its C (C_A) from the plant through $C_{Pl\text{-}A}$, and allocates it in order to respiration ($C_{Resp,A}$) and biomass ($C_{Struct,Pl}$) (Table 2). Additionally, half of the C in the arbuscules is recycled ($C_{Recyc,Arb}$), mimicking the presumed function of vesicles for nonstructural C storage (Smith & Read, 1997; Table 2). Fungal respiration ($C_{Resp,A}$) uses the same equation as plant respiration (Table 2). Structural C ($C_{Struct,A}$) is also similar, but it is limited by up to 20% of root mass (Table 2), so that the intraradical hyphae will not grow larger than the root containing them.

Table 3 Plant, arbuscular mycorrhizal fungal (AMF), microbial, and soil phosphorus equations, variables, and constants

Equation	Units
$I_{MaxP,A}$ =1.21e-7	g cm^{-2} d^{-1}
$I_{MaxP,Pl}$ = 1.21e-7	g cm^{-2} d^{-1}
$K_{MinP,A}$ =1.4e-5	g cm^{3}
$K_{MinP,Pl}$ = 1.4e-5	g cm^{3}
$P_A(t) = P_A(t - dt) + (P_{Uptake,A} + P_{Recyc,Arb} - P_{A\text{-}Pl} - P_{Struct,A})dt$ (initially $0.001Mass_A$)	g
$P_{A\text{-}Pl} = \min\{(0.0000003SA_{Arb})[(G_{Max,A} - 2C_A)/(G_{Max,A} - G_{Min,A})], (0.5P_{Uptake,A})\}P_{ExEff}$	g d^{-1}
$P_{Dep,A}(t) = P_{Dep,A}(t - dt) + (P_{Inflow,A} - P_{Uptake,A})dt$ (initially $(P_S/Vol_S)Vol_{Dep,P,A}$)	g
$P_{Dep,Pl}(t) = P_{Dep,Pl}(t - dt) + (P_{Inflow,P} - P_{Uptake,P})dt$ (initially $(P_S/Vol_S)Vol_{Dep,P,Pl}$)	g
P_{ExEff} varies from 0 to 1	unitless coefficient
$P_{Inflow,A} = P_S/V_S V_{Dep,P,A} - P_{Dep,A}$	g d^{-1}
$P_{Inflow,Pl} = ((P_S - P_{Dep,A} - P_{Uptake,M})/V_S V_{Dep,P,Pl} - P_{Dep,Pl})/T_S$	g d^{-1}
$P_M(t) = P_M(t - dt) + (P_{Uptake,M} - P_{Struct,M})dt$ (initially 0.0001)	g
$[P]_{Min,A} = 2.8e^{-10}$	g cm^{-3}
$[P]_{Min,Pl} = 2.8e^{-10}$	g cm^{-3}
$P_{Min,Hyp} = Mass_{Min,Hyp}/1000$	g d^{-1}
$P_{Min,M} = Mass_{Min,M}/120$	g d^{-1}
$P_{Min,Rt} = Mass_{Min,Rt}/1000$	g d^{-1}
$P_{Min,Sh} = Mass_{Min,Sh}/2000$	g d^{-1}
$P_{Pl}(t) = P_{Pl}(t - dt) + (P_{Uptake,Pl} + P_{A\text{-}Pl} + P_{Recyc,Pl} - P_{Struct,Pl})dt$ (initially $0.001Mass_{Pl}$)	g
$P_{Recyc,Arb} = Mass_{Senes,Arb}/2000$	g d^{-1}
$P_{Recyc,Sh} = Mass_{Senes,Sh}/2000$	g d^{-1}
$P_{Soil}(t) = P_{Soil}(t - dt) + (P_{Litter,Sh} + P_{Litter,Rt} + P_{Litter,Hyp} + P_{Senes,M} - P_{Uptake,Pl} - P_{Uptake,A} - P_{Uptake,M})dt$	g
$P_{Struct,A} = 0.001Mass_{RateIncr,A}$	g d^{-1}
$P_{Struct,M} = Mass_{RateIncr,M}/120$	g d^{-1}
$P_{Struct,Pl} = 0.001Mass_{RateIncr,Pl}$	g d^{-1}
$P_{Uptake,A} = SA_{Hyp}I_{MaxP,A}((P_{Dep,A}/V_{PDep,A}) - [P]_{Min,A})/(K_{MinP,A} + ((P_{Dep,A}/V_{PDep,A}) - [P]_{Min,A})) \times (1 - (1000P_A/Mass_A))$	g d^{-1}
$P_{Uptake,M} = If(CNRatio_M < 120)$ then 0 else $Mass_M/120$	g d^{-1}
$P_{Uptake,Pl} = SA_{Rt}I_{MaxP,Pl}((P_{Dep,Pl}/V_{PDep,Pl}) - [P]_{Min,Pl})/(K_{MinP,P} + ((P_{Dep,Pl}/V_{PDep,Pl}) - [P]_{Min,Pl})) \times (1 - (1000P_{Pl}/Mass_{Pl}))$	g d^{-1}
$Tort_S = 1.0$	unitless coefficient

Soil and microbial carbon Carbon enters the C soil pool (C_S) via mineralization of shoot, root and hyphal litter ($C_{Min,Sh}$, $C_{Min,Rt}$, $C_{Min,Hyp}$; Table 2). From there, C is taken up by the soil microbes ($C_{Uptake,M}$), which goes to microbial biomass and respiration. Ultimately, soil C is lost through microbial respiration ($C_{Resp,M}$). Microbial C uptake ($C_{Uptake,M}$) is 1.2 times the microbial biomass, to account for growth and respiratory needs (Table 2). Microbial C feeds to the nonstructural microbial C pool (C_M) (Table 2). In addition to the $C_{Uptake,M}$ inflow, C_M also receives recycled C from consuming microbial biomass for respiration ($C_{Senes,M}$) when C_S is insufficient (Table 2). Microbes lose nonstructural C to microbial respiration ($C_{Resp,M}$) and to incorporation in microbial biomass ($C_{Struct,M}$) (Table 2).

Phosphorus and nitrogen

The nutrient cycles for N and P use similar equations. Because of this, the P cycle is discussed in some detail, while the N cycle is described based on its differences from the P cycle.

Plant phosphorus For simplicity's sake, the movement of nutrients from the soil volume into the depletion zone is based on ratios. The soil nutrients (such as soil P, P_S) are assumed to be uniformly distributed within Vol_S, and this uniform concentration simply goes up or down as nutrients are added to or removed from the soil. Nutrients move into the depletion zone (for the plant, $P_{Dep,Pl}$; Table 3), based on volumetric concentration and soil tortuosity ($Tort_S$, Table 3).

$Tort_S$ is a soil tortuosity term that can be used to slow down nutrient movement and simulate a clay soil, but in this paper $Tort_S = 1$, so that part of the equation can be ignored.

The plant obtains P (P_{Pl}) through uptake from the soil P pool ($P_{Uptake,Pl}$), and as transfer from AMF ($P_{A\text{-}PL}$) (Table 3). It recycles half of the P bound in shoot biomass when it senesces ($P_{Recyc,Pl}$), while the nonstructural P pool decreases through incorporation to plant biomass ($P_{Struct,Pl}$) (Table 3). The P transferred from the fungus to the plant is described in the next section, and the other three equations are described below.

The equation for P uptake from the soil ($P_{Uptake,Pl}$) is a variation of a Michaelis–Menten equation (Lambers *et al.*, 1998; Table 3). In the first half of the equation, SA_{Root} is root surface area, $I_{MaxP,Pl}$ is the maximum P-inflow rate for plant roots, $P_{Dep,P}$ is the amount of P in the depletion zone around the root, $V_{Dep,P}$ is the volume of the P depletion zone, $[P]_{Min,Pl}$ is the P concentration at which uptake is zero, and $K_{MinP,Pl}$ is the P concentration at which the uptake rate is half maximum (Table 3). In the second half of the equation, P_{Pl} is the amount of nonstructural P in the plant, and $Mass_{Pl}$ is plant mass. This second half is a logistic equation that acts as an uptake cut-off when nonstructural P becomes equal to P in plant biomass (Table 3).

Plants are known to recycle a substantial portion of P and N from their leaves before shedding them (Lambers *et al.*, 1998). In this model, half the P from senescing shoots is recycled ($P_{Recyc,Pl}$; Table 3). There is no evidence that roots similarly recycle their nutrients back into the plant, so all root nutrients cycle instead to the soil.

Plant structural P ($P_{Struct,Pl}$) and structural C ($P_{Struct,C}$) equations are similar (Tables 2, 3). Any P not used to make biomass remains in the nonstructural pool (P_{Pl}).

AMF phosphorus The AMF P-cycling equations (P_A, $P_{Uptake,A}$, $P_{Struct,A}$, $P_{Recyc,Arb}$; Table 3) are very similar to those for plant P cycling. However, transfer of P from the fungus to the plant ($P_{A\text{-}PL}$) (Table 3) differs from the C-exchange term ($C_{PL\text{-}A}$). There is a difference between P and C exchange because AMF are obligate biotrophs, acquiring the entire C from their phototrophic partners (Smith & Read, 1997). Unlike the plant, the fungus cannot avoid transferring P unless P_{ExEff} is zero. Because of this, the model assumes that up to half the P taken from the soil can be transferred to the plant, limited by the surface area of the arbuscules through which the P is flowing and measured maximum transfer rates for the P.

Soil and microbial phosphorus The flow of P in the soil is similar to C, except that the P cycle is closed in the model, whereas the C cycle is open. As with C_S, inputs to the P soil pool (P_S) are P from depolymerized plant, AMF and microbial litter ($P_{Min,Sh}$, $P_{Min,R}$, $P_{Min,Hyp}$, $P_{Min,M}$, respectively), while plant, fungus and microbes take P out ($P_{Uptake,Pl}$, $P_{Uptake,A}$, $P_{Uptake,M}$; Table 3).

Microbial P is lost into the soil ($P_{Min,Pl}$) as 1/120th of senesced biomass ($Mass_{Senes,M}$) (Table 3). Whereas $C_{Senes,M}$ rejoins the microbial C pool for respiration, $P_{Senes,M}$ is shed into the soil (Table 3). Unlike plants, microbes have no mechanism for recycling P or N in this model.

Finally, $P_{Uptake,M}$ is modeled with a logical statement (Table 3). It is based on the assumption that microbial growth occurs only when the microbial C : N ratio > 12, otherwise growth is 0.

Plant and AMF nitrogen Fundamentally, N flows and pools in plant and AMF follow the same equations, which are very similar to those for P. Nitrogen is taken up from the soil into a nonstructural pool, from which biomass is formed. When the shoot or arbuscular biomass senesces, half is recycled back into the nonstructural pool, while none of the root or soil hyphal biomass is recycled. The N-recycling terms ($N_{Recycle,Pl}$, $N_{Recycle,A}$) differ slightly from the P-recycling term, because N is 10 times more abundant than P in biomass, for both plant and AMF. The only major difference between N and P models for plant and AMF is that both compete for the soil N pool, and do not exchange this element (Table 4).

Soil and microbial nitrogen Nitrogen in the soil (N_S) is very similar to P in the soil: it is shed from depolymerized litter and taken up by plant, AMF and microbes. As with microbial P uptake, $N_{Uptake,M}$ is simulated with a logical statement: microbes have to take up substantial C to survive. If N gets relatively too high (C : N < 12), then the microbes stop taking up N until the C : N reaches 12 again. Otherwise, N uptake is a function of microbial mass ($Mass_M$). As with P, the N in senescing microbial biomass is shed back into the soil (as $Mass_{Senes,M}/12$) rather than being recycled within the microbes (Table 4).

Methods

Parameterizing and testing the model

Parameterizing the model Some variables were determined through running the model, but many of the numbers were built in. One of the challenges in creating this model was that there is no single data set containing complete physiological information on an integrated plant/AMF/soil microbial system. Thus the simulation was a chimera of data from unrelated sets. Photosynthetic variables (including RGR_{Pl}) came from Poorter *et al.* (1990), while I_{Max}, K_{Min} and other nutrient-uptake parameters were taken from Craine *et al.* (2005). The latter were also used for the AMF, as they were roughly consistent with observations (Ezawa *et al.*, 2002). AMF hyphal diameter, arbuscule surface area and other data were taken from Smith & Read (1997 and references cited therein), and nutrient movement in the soil was from Nye & Tinker (1977). Other variables were determined arbitrarily for lack of data. Plant

Table 4 Plant, arbuscular mycorrhizal fungal (AMF), microbial, and soil nitrogen equations, variables, and constants

Equation	Units
$I_{MaxN,A} = 1.21e\text{-}5$	g cm^{-2} d^{-1}
$I_{MaxN,Pl} = 1.21e\text{-}5$	g cm^{-2} d^{-1}
$K_{MinN,A} = 1.4e\text{-}5$	g cm^3
$K_{MinN,Pl} = 1.4e\text{-}5$	g cm^3
$N_A(t) = N_A(t - dt) + (N_{Uptake,A} - N_{Recyc,Arb} - N_{Struct,A})dt$ (initially set at $0.01Mass_{AMF}$)	g
$N_{Dep,A}(t) = N_{Dep,A}(t - dt) + (N_{Inflow,A} - N_{Uptake,A})dt$ (initially $N_S/Vol_S)Vol_{Dep,N,A}$	g
$N_{Dep,Pl}(t) = N_{Dep,Pl}(t - dt) + (N_{Inflow,Pl} - N_{Uptake,Pl})dt$ (initially $N_S/Vol_S)Vol_{Dep,N,P}$	g
$N_{Inflow,A} = (N_S/V_S)V_{Dep,N,A} - N_{Dep,A}$	g d^{-1}
$N_{Inflow,Pl} = ((N_S - N_{Dep,A} - N_{Inflow,M})/V_S V_{Dep,N,Pl} - N_{Dep,Pl})Tort_S$	g d^{-1}
$N_M(t) = N_m(t - dt) + (N_{Uptake,M} - N_{Struct,M})dt$ (initially set at 0.0001)	g
$[N]_{Min,A} = 2.8e\text{-}8$	g cm^{-3}
$[N]_{Min,Hyp} = Mass_{Min,Hyp}/100$	g d^{-1}
$N_{Min,M} = Mass_{Min,Mt}/12$	g d^{-1}
$[N]_{Min,Pl} = 2.8e\text{-}8$	g cm^{-3}
$N_{Min,Rt} = Mass_{Min,Rt}/100$	g d^{-1}
$N_{Min,Sh} = Mass_{Min,Sh}/200$	g d^{-1}
$N_{Pl}(t) = N_{Pl}(t - dt) + (N_{Uptake,Pl} + N_{Recyc,Sh} - N_{Struct,Pl})dt$ (initially $0.01Mass_{Pl}$)	g
$N_{Recyc,Arb} = Mass_{Senes,Arb}/200$	g d^{-1}
$N_{Recyc,Sh} = Mass_{Senes,Sh}/200$	g d^{-1}
$N_{Soil}(t) = N_{Soil}(t - dt) + (N_{Litter,Sh} + N_{Litter,Rt} + N_{Litter,Hyp} + N_{Senes,M} - N_{Uptake,Pl} - N_{Uptake,A} - N_{Uptake,M})dt$	g
$N_{Struct,A} = 0.01Mass_{Incr,A}$	g d^{-1}
$N_{Struct,M} = Mass_{Incr,M}/12$	g d^{-1}
$N_{Struct,Pl} = 0.01Mass_{Incr,P}$	g d^{-1}
$N_{Uptake,A} = SA_{Arb}I_{MaxN,A}((N_{Dep,A}/V_{NDep,A}) - [N]_{Min,A})/(K_{MinN,A} + ((N_{Dep,A}/V_{Dep,A}) - [N]_{Min,A})) \times (1 - (100N_A/Mass_A))$	g d^{-1}
$N_{Uptake,M} = If(CNRatio_M < 12)$ then 0 else $Mass_M/12$	g d^{-1}
$N_{Uptake,Pl} = SA_{Rt}I_{MaxN,Pl}((N_{Dep,Pl}/V_{NDep,Pl}) - [N]_{Min,Pl})/(K_{MinN,Pl} + ((N_{Dep,Pl}/V_{Dep,Pl}) - [N]_{Min,Pl})) \times (1 - (100N_{Pl}/Mass_{Pl}))$	g d^{-1}

initial biomass was set at 0.2 g; for AMF it was 0.002 g. Microbial growth and decomposition rates were set arbitrarily at 0.2 and 0.8 g g^{-1}. The amount of N and P in the soil was determined empirically, as described in the following section. Note that none of these parameters was taken from Klironomos (2003), to prevent the test from being circular.

Testing the model The model was tested in two ways. First, N_S and P_S were varied systematically to determine whether plant growth would be nutrient-limited. From these results, an N_S and P_S were chosen that most closely matched Klironomos's (2003) experiments. Second, using this (N_S, P_S), we systematically varied C_{ExEff} and P_{ExEff} to determine if the simulation would show the variation in mycorrhizal interactions discovered by Klironomos (2003).

Results from (C_{ExEff}, P_{ExEff}) runs of (1, 1) and (0, 0) at a particular (N_S, P_S) were used to simulate the response of mycorrhizal (1, 1) and nonmycorrhizal (0, 0) systems to the same environmental variables. One fundamental hurdle for the model was for the (1, 1) plant to grow larger than the (0, 0) plant under some conditions. Initial N_S and P_S were systematically varied exponentially by six orders of magnitude, from 0.1 to 10 000 g (N) and from 0.01 to 1000 g (P) in 10 000 cm^3 soil. All 36 combinations of N and P were tested, each combination being one order of magnitude (0.1, 0.01). The proportional difference between mycorrhizal and nonmycorrhizal response to (N_S, P_S) was calculated as ((1, 1) – (0, 0))/(0, 0). Models were run for 365 d, using one day as the time step.

The criteria for choosing N_S and P_S for the second test were that the proportional difference should be as close to 50% as possible (Klironomos, 2003). Second, if possible N_S should be *c.* 1–100 mg kg^{-1} and P_S around 0.1–10 mg kg^{-1}, to match Klironomos's system. Once N_S and P_S had been chosen, these values were used for the next test.

To test the model plausibility, C_{ExEff} and P_{ExEff} were varied independently from 0 to 1 in 0.1 increments, for 121 separate runs. Plant and AMF weight after 365 d were plotted, and proportional differences between these values and the weights for (0, 0) control were calculated and tallied. This resulted in growth surfaces showing biomass as a function of particular (C_{ExEff}, P_{ExEff}) combinations. The proportional differences

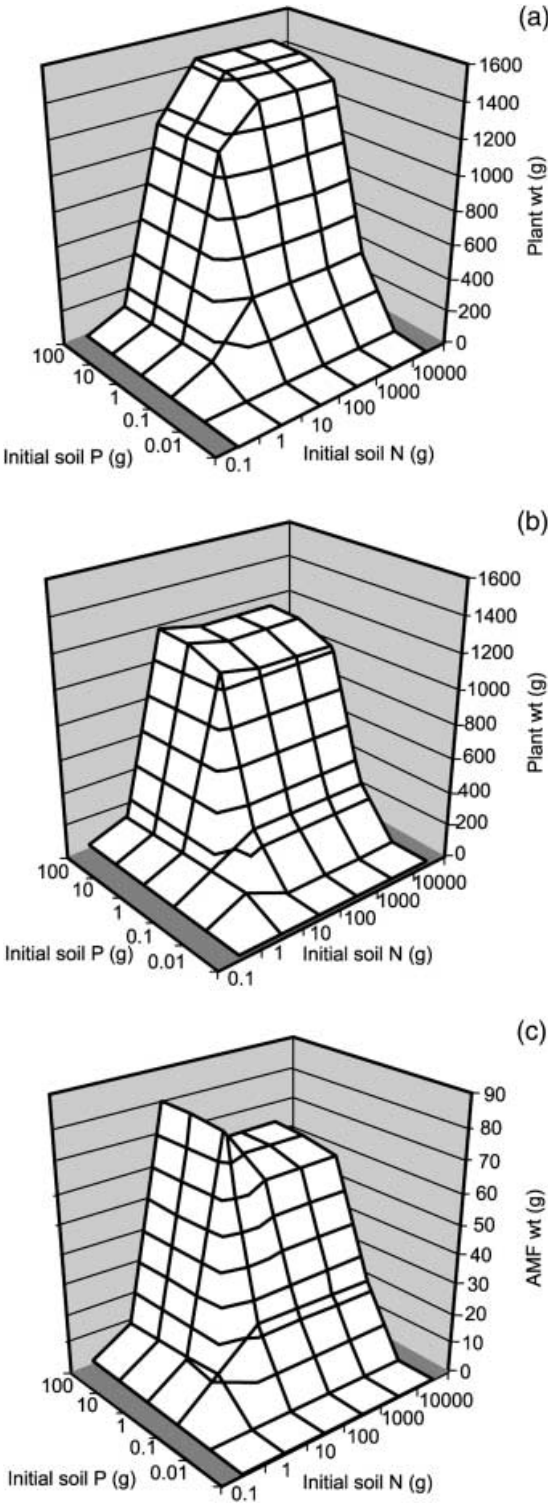

Fig. 2 Response surfaces showing effects of systematically varying initial soil nitrogen from 0.1 to 10 000 g and phosphorus from 0.01 to 1000 g. (a) Plant weight after 365 d with (C_{ExEff}, P_{ExEff}) (1, 1); (b) plant weight after 365 d at (0, 0); (c) arbuscular mycorrhizal fungal (AMF) weight after 365 d at (1, 1).

Table 5 Plant proportional weight differences under different initial nitrogen (N) and phosphorus (P) amounts. Proportional weight differences, shown as percentages, were calculated as explained in the text

Initial P (g)	Initial N (g) 0.1	1	10	100	1000	10 000
0.01	1.4%	1.3%	1.3%	1.3%	1.3%	1.3%
0.1	40.2%	–99.4%	38.1%	38.1%	38.1%	38.1%
1	6.6%	–3.0%	43.6%	43.6%	43.6%	43.6%
10	–0.7%	–8.8%	4.5%	29.3%	29.3%	29.3%
100	–0.8%	–10.7%	–3.2%	27.9%	27.9%	27.9%
1000	–1.1%	–9.2%	–3.4%	27.7%	27.7%	27.7%

were compared with those of Klironomos (2003), which show a proportional biomass response with a mean of 0 ± 50%; in other words, mycorrhizal plants were, on average, the same size as nonmycorrhizal ones, and could be anywhere from half to double that size (Klironomos, 2003).

Results

Response to variation in N_S and P_S

Both plant and AMF showed nutrient limitation at low N and P levels (Fig. 2), and for *c.* 72% of N and P combinations the 'mycorrhizal' (1, 1) plant was larger than the 'nonmycorrhizal' (0, 0) plant (Fig. 2). The response surface showed interesting complexities. For instance, both the nonmycorrhizal plant and AMF showed the largest weight at an initial N = 10 g and P > 10 g, while the mycorrhizal plant was largest at N > 10 g and P > 10 g (Fig. 2). These responses were probably caused by competition for N among the organisms. In this setup, it was also interesting that the AMF had a beneficial effect on plant growth even when N and P were not strongly limiting.

Following the criteria above, the closest fit to Klironomos's setup was found at P_S = 1 g and N_S > 10 g (Table 5), where there was a 43.6% proportional difference. As N_S = 10 g and P_S = 1 g were close to Klironomos's real running conditions, these soil nutrient levels were used to test the plausibility of the model. Note that this is not a circular test, as Klironomos (2003) was not used for any of the physiological parameters used to construct the model.

Response to variation in C_{ExEff} and P_{ExEff}

The response of plant and AMF showed a mix of expected, hoped-for and unexpected responses to variations in C_{ExEff} and P_{ExEff}. First, as expected, when no C was exchanged, the system was effectively nonmycorrhizal. Plant weights differed by approx. 0.6 g along the C = 0.0 isocline no matter what P_{ExEff} was used, and AMF failed to grow (Fig. 3). However, any C flow (C_{ExEff} > 0) resulted in AMF growth (Fig. 3).

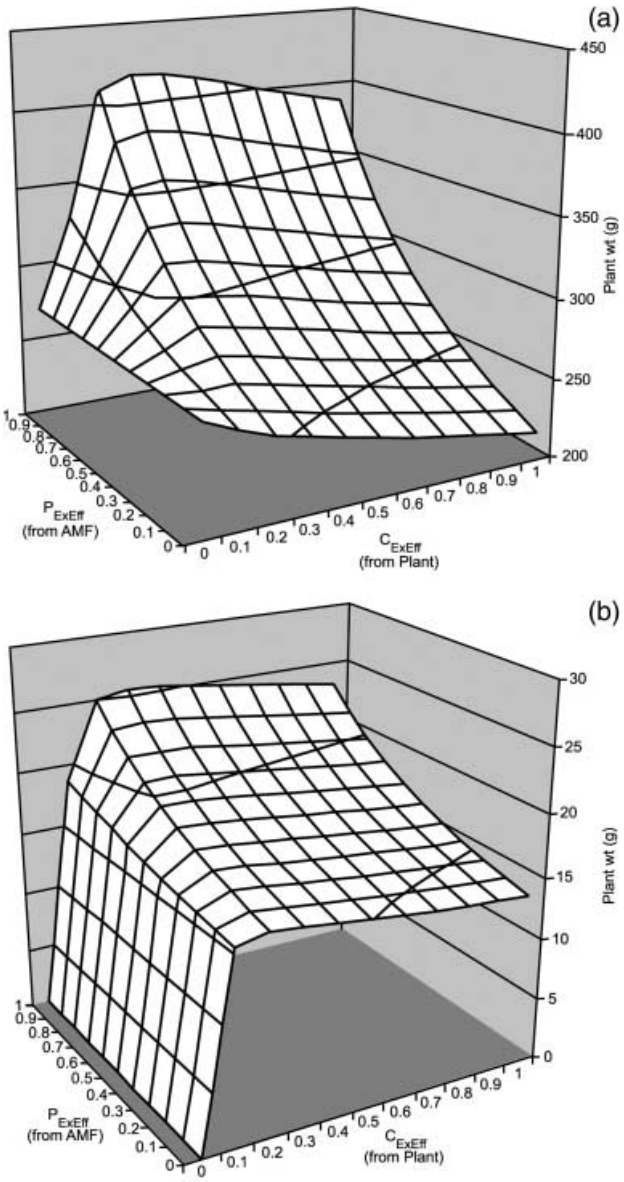

Fig. 3 Response surfaces showing effects of varying C_{ExEff} and P_{ExEff} between 0 and 1, using initial soil nitrogen of 10 g and phosphorus of 1 g. (a) Plant weight after 365 d; (b) arbuscular mycorrhizal fungal (AMF) weight after 365 d.

In approx. 34% of cases the fungus effectively parasitized the plant, reducing plant weight to less than the nonmycorrhizal state (269 g) in a triangular area stretching from (0, 0) to (1, 0.5) (Fig. 3). The minimum plant weight, 217.8 g, occurred at (1, 0), where plant C flowed freely, but no P flowed from the fungus (Fig. 3).

The two hoped-for results were the tests against Klironomos's data. First, the range of plant weight responses was 81–155%, relative to the nonmycorrhizal (0, 0) weight (Fig. 4). Second, the tally of responses was close to a normal curve, with a mean response of approx. 10%, rather than 0% as predicted, and missing 20% of the negative side.

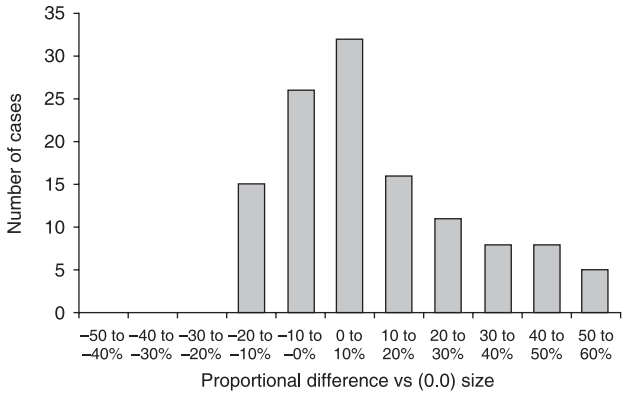

Fig. 4 Tally of proportional differences in plant weights. Data are from Fig. 3a, reformatted as proportional difference between a weight and the (0, 0) point, and tallied in 10% increments.

The major unexpected result was that (1, 1) was not the largest plant or AMF. For both plant and AMF, the combination that produced the largest biomass was (0.3, 1), where the plant weighed 416.86 g and the AMF 25.38 g (Fig. 3). In this model, it was possible for the plant to act inefficiently, transferring more C than necessary, and allocating more biomass to shoot than root. This inefficiency occurred above (0.3, 1). AMF biomass declined in a similar way, largely because arbuscular biomass was limited by root size. While this result was unexpected, it meant that the maximum proportional plant size was roughly the same as that seen in Klironomos's work.

Discussion

The fit between Klironomos's data and the model output was not perfect, but it was sufficiently good to render the model plausible. The chief defect of the model, in its current formulation, was that the fungus could not parasitize the plant sufficiently. To replicate Klironomos's results perfectly, the fungus would have had to reduce plant weight by 50% under some conditions. Given that Klironomos's data were from dozens of plant and AMF species, while this model was a chimera of numerical values from multiple sources, the match between model and reality was surprisingly good.

In this study, it is important to remember that the model was parameterized using (C_{ExEff}, P_{ExEff}) of only (1, 1) and (0, 0). Neither parameter was a maximum nor a minimum, so the maxima, minima, response curves and AMF parasitism truly tested the model, and the test was not circular. While it is possible that the match between model and data is coincidence, it is more likely this model of nutrient transfers has some biological validity.

If we accept the model as plausible, it has implications both for mycorrhizal biology and for the theory of symbioses. For mycorrhizologists, the model predicts that C and P transfers are unlinked, and this will be tested as the molecular

mechanisms of AM nutrient transfer are described in detail. Additionally this model suggests that the ecotypic variation described by Klironomos (2003) could be caused by allelic diversity in the genes underlying mycorrhiza formation, and to the mismatch between the genes products of different symbiont ecotypes. Researchers are elucidating the plant genes responsible for AM nutrient transfer (Harrison, 2005; Javot *et al*., 2007). Their work is exciting, but it is still in an early stage, describing the genes necessary in the symbiosis rather than the diversity of interactions. However, it is heartening to note that the model appears to produce results consistent with those of Javot *et al*. (2007) and perhaps others (unpublished data).

Second, and more importantly, the model demonstrates that investment in the search for nutrients is an important conceptual model for understanding mycorrhizal nutrient exchanges. Currently, most models (such as Fitter, 2006) are predicated on the notion that the instantaneous exchange of C and P is the important frame of analysis, and questions have focused on how partners may benefit or be cheated in an exchange. In contrast, we suggest that C and P fluxes within mycorrhizas are as much investments as instantaneous exchanges, and that the economic mathematics of risk, rate of return, and return on investment apply to mycorrhizal interactions. This opens up a rich new country for theorists, as the mathematics of investments are well developed but have not been applied to mycorrhizal interactions in any comprehensive way.

In summary, it is possible to explain much, but not all, of the variation seen in plant–AMF interactions through a simple model of nutrient exchange. In this model, nutrient transfers are independent of each other, and it is possible for nutrient flows to be nonexistent, adequate or excessive, resulting in either mutualistic or parasitic interactions. These results came from only one iteration of this particular model, and the model can easily be adapted to study other phenomena, such as variation in organ lifespan, N exchange by mycorrhizas, litter exploitation (reimagining the AMF as an ectomycorrhizal fungus), or interactions among multiple plants and fungi with varying properties. This model is an adaptable tool, and we hope that others will use it freely.

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References

Brady NC, Weil RR. 2001. *The nature and properties of soils*. New York: Prentice Hall.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004. Toward a metabolic theory of ecology. *Ecology* **85**: 1771–1789.

Chapin FS. 1991. Effects of multiple environmental stresses on nutrient availability and use. In: Mooney HA, Winner WE, Pell EJ, eds. *Response of plants to multiple stresses*. San Diego, CA, USA: Academic Press, 67–88.

Clarkson DT. 1981. Nutrient interception and transport by root systems. In: Johnson CB, ed. *Physiological factors limiting plant productivity*. London: Butterworths, 307–314.

Craine JM, Fargione J, Sugita S. 2005. Supply pre-emption, not concentration reduction, is the mechanism of competition for nutrients. *New Phytologist* **166**: 934–940.

Ezawa T, Smith SE, Smith FA. 2002. P metabolism and transport in AM fungi. *Plant and Soil* **244**: 221–230.

Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* **172**: 3–6.

Harrison MJ. 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* **59**: 19–42.

Harrison MJ, Dewbre GR, Liu JY. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisiton of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* **14**: 2413–2429.

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **104**: 1720–1725.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–586.

Klironomos JN. 2003. Variation in plant response to native and exotic arbuscular mycorhizal fungi. *Ecology* **84**: 2292–2301.

Lambers H, Atkin O, Scheurwater I. 1996. Respiratory patterns in roots in relation to their functioning. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots, the hidden half*. New York: Marcel Dekker, 323–362.

Lambers H, Chapin FS, Pons TL. 1998. *Plant physiological ecology*. New York: Springer-Verlag.

Leigh RA, Wyn-Jones RG. 1985. Cellular compartmentation in plant nutrition: the selective cytoplasm and the promiscuous vacuole. In: Tinker B, Lauchli A, eds. *Advances in plant nutrition*. New York: Praeger Scientific, 249–277.

Maeda D, Ashida K, Iguchi K, Svetlana C, Deguchi Y, Izui K, Hata S. 2005. Identification of an arbuscular mycorrhiza-specific phosphate transporter gene and characterization of its RNAi knockdown transformants. *Plant and Cell Physiology* **46**: S126–S126.

Maeda D, Ashida K, Iguchi K, Chechetka SA, Hijikata A, Okusako Y, Deguchi Y, Izui K, Hata S. 2006. Knockdown of an arbuscular mycorrhiza-inducible phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis. *Plant and Cell Physiology* **47**: 807–817.

Nagy R, Karandashov V, Chague W, Kalinkevich K, Tamasloukht M, Xu GH, Jakobsen I, Levy AA, Amrhein N, Bucher M. 2005. The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *Plant Journal* **42**: 236–250.

Nagy R, Vasconcelos MJV, Zhao S, McElver J, Bruce W, Amrhein N, Raghothama KG, Bucher M. 2006. Differential regulation of five Pht1 phosphate transporters from maize (*Zea mays* L.). *Plant Biology* **8**: 186–197.

Nye PH, Tinker PB. 1977. *Solute movement in the soil–root system*. Berkeley, CA, USA: University of California Press.

Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **99**: 13324–13329.

Poorter H, Remkes C, Lambers H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology* **94**: 621–627.

Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* **414**: 462–466.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. London: Academic Press.

Sterner RW, Elser JJ. 2002. *Ecological stoichiometry*. Princeton, NJ, USA: Princeton University Press.

Thornley JHM. 1995. Shoot : root allocation with respect to C, N, and P: an investigation and comparison of resistance and teleonomic models. *Annals of Botany* **75**: 391–405.

Thornley JHM. 1997. Modelling allocation with transport/conversion processes. *Silva Fennica* **31**: 341–355.

Verhoeven JTA, Koerselman W, Meuleman AFM. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology and Evolution* **11**: 494–497.

Versaw WK, Chiou TJ, Harrison MJ. 2002. Phosphate transporters of *Medicago truncatula* and arbuscular mycorrhizal fungi. *Plant and Soil* **244**: 239–245.